CLAIMS

We Claim:

1. An isolated neublastin nucleic acid comprising the sequence of any one of SEQ. ID. NOS. 1, 3, 8, 13, 14, 15, 29 and 30.

2. A nucleic acid sequence comprising an open reading frame which encodes neublastin neurotrophic factor or a unique subregion thereof, and which codes on expression for a neublastin polypeptide which comprises at least 70% homology to SEQ. ID. NOS. 2, 4, 5, 6, 7, 9, 10, 11, 12, and 16.

3. A nucleic acid that hybridizes specifically under high stringency solution hybridization conditions to the nucleic acid of claim 1 or 2.

4. A nucleic acid that comprises a nucleic acid sequence that is complementary to the nucleic acid of claim 3.

5. A method of using a nucleic acid of any one of claims 1-4, comprising the step of causing a polypeptide encoded by said nucleic acid to be expressed in a cell.

6. The method of claim 5, further comprising the step of administering said nucleic acid to an animal, and causing said polypeptide to be expressed in said animal.

7. A vector comprising the nucleic acid of any one of claims 1-4.

8. The vector of claim 7, wherein said vector is an expression vector.

9. A method of using the vector of claim 8, comprising the step of causing a polypeptide encoded by said nucleic acid to be expressed from said nucleic acid.

10. A cell transformed with the nucleic acid of any one of claims 1-4.

11. The cell of claim 10, wherein said cell is selected from the group consisting of mammalian cells, fungal cells, yeast cells, insect cells and bacterial cells.

12. The method of claim 11, wherein said cell is a Chinese hamster ovary cell.

13. The method of claim 11, wherein said cell is a cell derived from the mammalian central nervous system.

14. A neublastin neurotrophic factor polypeptide comprising any one of the amino acid sequences set forth in SEQ. ID. NOS. 2, 4, 5, 6, 7, 9, 10, 11, 12 and 16.

15. The polypeptide of claim 14, wherein said polypeptide is glycosylated.

16. The polypeptide of claim 14, wherein said polypeptide is coded for by a nucleic acid of any one of claims 1-4.

17. A method of making the polypeptide of any one of claims 14-16, said method comprising the step of expressing said polypeptide from a neublastin neurotrophic factor nucleic acid.

18. The method of claim 17, comprising the step of culturing a cell comprising said neublastin neurotrophic factor nucleic acid in a culture medium which permits the production of said polypeptide.

19. The method of claim 18, further comprising the step of recovering said polypeptide from said culture medium.

20. A purified polypeptide obtained by the method of claim 19.

21. A composition comprising the polypeptide of any one of claims 14-16 and 20, and a pharmaceutically acceptable carrier.

22. A polypeptide having an amino acid sequence which is at least 90% homologous to any one of the sequences set forth in SEQ. ID. NOS. 2, 4, 5, 6, 7, 9, 10, 11, 12 and 16.

23. A method of administering the polypeptide of any one of claims 14-16 and 20, comprising the step of delivering said polypeptide to an in vitro cell culture or in vivo to a mammal.

24. The method of claim 23, wherein said administration comprises systemic administration.

25. The method of claim 23, wherein said mammal is afflicted with a condition selected from the group consisting of cerebral ischaemic neuronal damage, traumatic brain injury, peripheral neuropathy, Alzheimer's disease, Huntington's disease, Parkinson's disease, amyotrophic lateral sclerosis, and memory impairment.

26. The method of claim 23, wherein said mammal is afflicted with a neuronal disorder of the peripheral nervous system, the medulla, or the spinal cord.

27. A method of treating a neurodegenerative disease or disorder in an animal, comprising administering to said animal one or more of the neublastin nucleic acids set forth in SEQ. ID. NOS. 1, 3, 8, 13, 14, 15, 29 and 30.

28. A method of treating a neurodegenerative disease or disorder in an animal, comprising administering to said animal a neublastin polypeptide one or more of the neublastin polypeptides set forth in SEQ. ID. NOS. 2, 4, 5, 6, 7, 9, 10, 11, 12 and 16.

29. An antibody that binds to any one of the polypeptides set forth in SEQ. ID. NOS. 2, 4, 5, 6, 7, 9, 10, 11, 12 and 16.

30. The antibody of claim 29, wherein said antibody is a monoclonal antibody.

31. A method of determining whether a neurodegenerative disease or disorder in an animal is associated with an altered activity in a neublastin neurotrophic factor polypeptide, said method comprising the steps of:

contacting a biological sample from said animal with the antibody of claims 29 or 30, and

determining whether an immune complex forms between said antibody and said protein as an indication of whether said neural condition results from an altered level of activity in said neublastin neurotrophic factor polypeptide.

32. The method of claim 31, further comprising the step of comparing a level of said immune complex that forms in said sample with a level of said immune complex that forms in a corresponding biological sample from a patient lacking said neural condition, and determining from said comparison whether said disease or disorder results from said abnormality in a neublastin neurotrophic factor polypeptide.

33. A nucleic acid comprising any one of the sequences set forth in SEQ. ID. NOS. 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 31 and 32.

34. A method of producing any one of the polypeptides set forth in set forth in SEQ. ID. NOS. 2, 4, 5, 6, 7, 9, 10, 11, 12 and 16, comprising culturing a cell that contains any one of the nucleic acid sequences set forth in SEQ. ID. NOS. 1, 3, 8, 13, 14, 15 29 and 30 under conditions permitting the production of the polypeptide, and recovering the polypeptide from the culture medium.

35. A method for producing a neublastin polypeptide comprising:

(a) introducing a polynucleotide which codes on expression for a neublastin polypeptide into a cell or introducing a regulatory sequence by homologous recombination into a cell, such that the regulatory sequence regulates expression of an endogenous neublastin gene, to make a neublastin production cell;

(b) culturing the neublastin production cell under culture conditions which result in expression of a neublastin polypeptide.

36. A neublastin polypeptide comprising:

(a) conserved Cys residues;

(b) at least 70% homology to any one of the sequences set forth in SEQ. ID. NOS. 2, 4, 5, 6, 7, 9, 10, 11, 12, and 16,

wherein said neublastin polypeptide exhibits neurotrophic activity.

37. The neublastin polypeptide of claim 36, wherein the polypeptide has a C-terminal amino acid sequence as set forth in AA_{72}-AA_{105} of SEQ. ID. NO. 2.

38. The neublastin polypeptide of claim 36 wherein the polypeptide has a C-terminal amino acid sequence as set forth in AA_{41}-AA_{105} of SEQ. ID. NO. 2

39. The neublastin polypeptide of any one of claims 36, 37 or 38, wherein said homology to any one of the sequences set forth in SEQ. ID. NOS. 2, 4, 5, 6, 7, 9, 10, 11, 12, and 16 is greater than 85%.

40. The neublastin polypeptide of any one of claims 36, 37 or 38, wherein said homology to any one of the sequences set forth in SEQ. ID. NOS. 2, 4, 5, 6, 7, 9, 10, 11, 12, and 16 is greater than 95%.

41. A neublastin polypeptide of any one of SEQ. ID. NOS. 2, 4, 5, 6, 7, 9, 10, 11, 12, and 16, and variants thereof with conservative amino acid substitutions.

42. The neublastin polypeptide of claim 41 wherein the conservative amino acid substitutions represent less than 10% of the total number of residues in the polypeptide.

43. The neublastin polypeptide of claim 41 wherein the conservative amino acid substitutions represent less than 2% of the polypeptide.

44. The neublastin polypeptide of claim 41 wherein the conservative amino acid substitutions represent a single amino acid substitution in the mature sequence, wherein the both the substituted and replacement amino acid are non-cyclic.

45. An isolated nucleic acid sequence as set forth in any one of SEQ. ID NOS. 17-28 and 31-32.

46. An isolated nucleic acid sequence comprising nucleotides 721 - 865 of SEQ.ID.NO. 1, nucleotides 718 - 861 of SEQ.ID.NO. 3, nucleotides 718 - 861 of SEQ.ID.NO. 8, and nucleotides 1647 - 2136 of SEQ.ID.NO. 15.

47. An isolated nucleic acid sequence consisting of between 10-25 contiguous base pairs falling within or produced from any one of the sequences of claim 46.

48. An isolated nucleic acid sequence comprising nucleotides 1-10 of SEQ.ID.NO. 1, nucleotides 1 - 57 of SEQ.ID.NO. 8, nucleotides 1-974 of SEQ.ID.NO. 15

49. An isolated nucleic acid sequence consisting of between 10-25 contiguous base pairs falling within or produced from any one of the sequences of claim 48.

50. A method for identifying, isolating or amplifying a neublastin nucleic acid sequence comprising using the nucleic acids of any one of claims 45-49, 43, 44 or 45 as a primer or a probe.

51. A neublastin nucleic acid isolated by the method of claim 50.

52. An isolated nucleic acid sequence comprising the sequence set forth in SEQ. ID. NOS. 13 or 14.

53. A synthetic gene encoding a neublastin polypeptide, the synthetic gene as set forth in SEQ.ID.NOS. 29 or 30.

54. An antibody to a neublastin peptide or neublastin polypeptide, wherein said antibody is generated using any one of peptides:

GPGSRARAAGARGC (AA30-43 of SEQ ID NO:9);
LGHRSDELVRFRFC (AA 57-70 of SEQ ID NO:9);
CRRARSPHDLSL (AA 74-85 of SEQ ID NO:9);
LRPPPGSRPVSQPC (AA 94-107 of SEQ ID NO:9);
STWRTVDRLSATAC (AA 123-136 of SEQ ID NO:9).
CRLRSQLVPVRALGLGHRSDELVRFRFC (AA43-70 of SEQ. ID. NO: 9);
CRRARSPHDLSLASLLGAGALRPPPGSRPVSQPC (AA74-107 of SEQ. ID. NO: 9);
CRPTRYEAVSFMDVNSTWRTVDRLSATAC (AA108-136 of SEQ. ID. NO: 9);
CRPTRYEAVSFMDVNST (AA108-124 of SEQ ID NO: 9); and
ALRPPPGSRPVSQPC (AA93-107 of SEQ ID NO: 9).

55. The neublastin polypeptide of claim 36 wherein said polypeptide comprises seven cysteine residues conserved as set forth in SEQ ID NO:2 at positions 8, 35, 39, 72, 73, 101 and 103, or as in SEQ ID NOS: 4 and 9 at positions 43, 70, 74, 107, 108, 136 and 138.

56. A kit comprising, in one or more containers, a substance selected from the group consisting of a neublastin polypeptide, an antibody against a neublastin polypeptide, nucleic acid probes capable of hybridizing to RNA of neublastin, or pairs of nucleic acid primers capable of priming amplification of at least a portion of a neublastin gene.

57. A method of diagnosing or screening for the presence of or a predisposition for developing a disease or disorder characterized by an aberrant level of a neublastin polypeptide in a subject comprising measuring the level of said neublastin polypeptide, RNA encoding the

neublastin polypeptide, or functional activity of the neublastin polypeptide in a sample derived from the subject, in which an increase or decrease in the level of the neublastin polypeptide, neublastin RNA, or functional activity of neublastin polypeptide in the sample, relative to the level of the neublastin polypeptide, neublastin RNA or functional activity of neublastin found in an analogous sample not having the disease or disorder or a predisposition for developing the disease or disorder, indicates the presence of the disease or disorder or a predisposition for developing the disease or disorder.

58. A method for screening a purified neublastin polypeptide, or derivative or fragment thereof, or a modulator of the activity of the foregoing, for activity in treating or preventing a disease, comprising measuring and comparing alterations in the phenotype, genotype, behavior, survival or proliferation of cells from a cell line or test animal which are derived from or display characteristics associated with the disease, which cells or animals have been contacted with or administered the neublastin polypeptide, derivative, fragment, or modulator, with the phenotype, genotype, behavior, survival or proliferation in cells or animals not so contacted with or administered the neublastin polypeptide, derivative, fragment, or modulator.

59. A method of treating a neurological disorder selected from the group consisting of peripheral neuropathies in a mammal which comprises administering a therapeutically effective amount of a neublastin polypeptide, wherein said peripheral neuropathy is selected from the group consisting of trauma-induced neuropathies, chemotherapy-induced neuropathies, toxin-induced neuropathies, drug-induced neuropathies, vitamin-deficiency-induced neuropathies; idiopathic neuropathies; and diabetic neuropathies.

60. The method of claim 23 wherein the neublastin is delivered directly into the central nervous system.

61. The method of claim 23 wherein the neublastin is delivered systemically by subcutaneous injection, intravenous administration, or intravenous infusion. administration.

62. A method of using the sequence of one or more nucleic acids of any one of claims 1-4 or 45-49 in a computer program for identifying, isolating or detecting novel nucleic acid sequences.

63. A method of using the sequence of one or more nucleic acids of any one of claims 1-4, 33 or 45-49 on a fixed substrate or DNA chip for identifying, isolating or detecting novel nucleic acid sequences.

64. A method of using the sequence of one or more polypeptides of any one of claims 14-16, 20, 22, or 36-44 in a computer program for identifying, isolating or detecting novel nucleic acid sequences.

65. A method for identifying a candidate compound that induces a neuroblastin-mediated biological effect, the method comprising the steps of:

(a) providing a test cell, said cell when contacted with neublastin being induced to express a detectable product;

(b) exposing the cell to the candidate compound and detecting the detectable product, said expression of the detectable product indicating the ability of the candidate compound to induce said neuroblastin-mediated biological effect.